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DISCOVERY

Haematological and biochemical indices of albino rats fed processed sickle pod (*Senna obtusifolia*) seed meal based-diets

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A feeding trial was conducted to evaluate the effects of feeding raw or processed *Senna obtusifolia* seed meal based-diets on the haematological and biochemical indices of albino rats. Five experimental diets were compounded to contain 0 and 20% each of the raw, toasted, boiled and fermented *Senna obtusifolia* seed meal represented as T1, T2, T3, T4 and T5, respectively. A total of 60 young albino rats with average body weight of 72.67 g were randomly allotted to the 5 dietary treatments in complete randomized designed with 3 replicates of 4 rats each. Data were collected on chemical composition of raw and processed *Senna obtusifolia* seed meals packed cell volume (PCV), haemoglobin (Hb), Red blood cells (RBC), white blood cells (WBC), total protein, albumin, globulin, creatinine, aspartate aminotransferase, alanine aminotransferase and total bilirubin. The chemical composition of *Senna obtusifolia* seed meal revealed that the raw seed meal had crude protein of 22.68%. The different processing methods were observed to reduce the protein content of the seed meal except for the fermented seed meal which indicated an increase in protein content (23% CP). The PCV (29.67%), Hb (9.67g/dl) and RBC (2.90 x 10^3/mm^3) were significantly (P<0.05) depressed in the albino rats fed the raw *Senna obtusifolia* seed meal based-diet. However, WBC was observed to be high (6.77 x 10^6/mm^3) in the group of albino rats fed the raw *Senna obtusifolia* seed meal based-diets. Low total protein (3.54 g/dl), albumin (0.91 g/dl) and globulin (2.83 g/dl) were observed in threats fed raw *Senna obtusifolia* seed meal based-diets. Higher values for aspartate aminotransferase (47.33 µ/L) alanine aminotransferase (37.33 µ/L) and total bilirubin (16.67 mmol/L) were recorded in albino rats fed the raw *Senna obtusifolia* seed meal based-diets. Among the albino rats fed the processed *Senna obtusifolia* seed meal based-diets, those fed the fermented seed meal based-diets recorded better haematological and biochemical values. It was concluded that albino rats fed the fermented *Senna obtusifolia* seed meal indicated better heamatological and biochemical constituents and is therefore recommended for feeding albino rats.

INTRODUCTION

The problem of feeding domestic animals in Nigeria, can be addressed by utilizing cheaper alternative feed materials. Augustine *et al*. (2017a) reported that one of the ways to address the feed crises in the Nigerian livestock sub-sector is to encourage the use of lesser-known legumes in feeding livestock. One of such alternative wild legumes that has long been under-utilized is *Senna obtusifolia* which is an annual or biennial shrub growing up to 2.5 m tall, but usually less than 2 m in height. The seeds (3-6 mm long) are dark brown in colour, shiny in appearance with rhomboid or irregular shape (Lusweti, 2011). The raw seed was reported to contain good protein content (26.95 to 28.79% CP) but also contain some anti-nutritional factors such as tannins, oxalates and phytates (Augustine *et al*. 2018).

The effects of raw *Senna obtusifolia* seed meal based-diets on blood constituents of domestic chickens and albino rats were well documented by Augustine (2016) and Augustine *et al*. (2017b). The presence of these anti-nutritional factors in a diet may have the potentials for toxicity when consumed by an animal. It is important to note that a range of haematological and biochemical indices can be a useful tool for diagnosing and interpreting toxicological and safety studies especially in nutritional experiments (Mary and Charlse, 2008). In view of the above, it is imperative todetoxify the seeds before it can be safely fed to domestic animals. Therefore, it is important to evaluate the best processing method that will enhance optimal utilization of *Senna obtusifolia* seed meal with little or no adverse effects on the blood constituents of albino rats. This study was conducted to investigate the effects of feeding raw or processed *Senna obtusifolia* seed meal based-diets on blood parameters of albino rats.

MATERIALS AND METHODS

Location of the Study Area

The study was conducted at the Animal Research House in the Department of Biological Sciences, Adamawa State University, Mubi.

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The area lies between latitudes 9º30` and 11ºN of the equator and longitude 13ºand 13º45[1] (Adebayo, 2004). The dry season of the area commences early October and last up to April while the wet season begins from May and attains it peak at between July and August and declines in September. The average temperature and rainfall are 25.4ºC and 935mm (Climate-Data.Org, 2018))

Collection and Processing of Raw *Senna obtusifolia* Seeds

The test materials, (*Senna obtusifolia)* seeds were collected from the wild around Hong Local Government area of Adamawa State, Nigeria. The matured pods containing the seeds were harvested from the mature stands at the beginning of the dry season starting from October. The pods were properly sun-dried and threshed to obtain the seeds that were used for chemical analysis and the feeding trials. The seeds of *Senna obtusifolia* were divided into five (5) batches as follows: the first batch was left unprocessed (raw); second batch was toasted in a frying pan until the seeds changed to dark brown colour; third batch was boiled for one hour using an aluminum pot, the boiling time was taken when the water began to boil and fourth batch was boiled for one hour drained, washed, placed in an air-tight container and allowed to naturally ferment for seven days. Each of the sample was properly sun-dried and milled and were used for the chemical analysis and the feeding trial.

Chemical Analysis

The proximate composition of *Senna obtusifolia seeds* and the experimental dietswere determined using standard laboratory procedures of AOAC (2004). The Kjeldah procedure was used to determine the crude protein (CP) content. The dry matter was determined first by obtaining the moisture content using the vacuum oven-dried method. The dry matter was computed using the formula:

Dry matter = 100 - % moisture.

The ashing procedure as described by Onu (2005) was used to determine the ash content while the defatting and boiling methods were used to determine the crude fibre. The soxhletfat extraction method was used to determine the ether extract (EE). The nitrogen-free extract (NFE) was determined using the formula shown below:

NFE = 100 – (%moisture + CP + CF + EE + Ash)

The energy values of the seeds and the experimental diets were calculated using the formula of Pauzenga (1985): ME (kcal/kg) = 37 x % CP + 81 x % EE + 35.5 x % NFE. The level of the anti-nutritional factors and amino acid profile were determined using the chromatographic methods specifically (HPLC), Scientific BLC 10/11model as described by Pearson (1991).

Experimental Diets and Treatments

Five experimental diets were formulated to contain 0 and 20% each of the raw, toasted, boiled and fermented *Senna obtusifolia* seed meals. The diets were designated as T1, T2, T3, T4 and T5, respectively. The 0% *Senna obtusifolia* seed meal based diet served as the positive control while 20% raw SOSM served as the negative control (Table 1).

Experimental Animals and their Management

A total of sixty young albino rats with an average body weight of 72.67g were used in a 30-days feeding trial. The albino rats were managed in a constructed metal cages.Water and known quantity of feed were supplied *ad-libitum.*

Experimental Design

Sixty albino rats with average weight of 72.67 g were randomly allocated to the five (5) dietary treatments in a completely randomized design with three (3) replicates of four (4) rats each.

Collection of Blood Samples

Three (3) rats were randomly selected from each replicate cage and blood samples were collected immediately after decapitation. About 5 ml each of the blood sample was collected into a test tube containing Ethylene diamine tetraacetate (EDTA) and was analyzed for haematological parameters. The blood samples for biochemical analyses on the other hand, were collected into an EDTA-free test tubes and were allowed to clot for serum separation. Serum was separated from the plasma immediately by centrifugation of the blood at 4000 rpm for 15 minutes and thereafter quickly taken to the Laboratory for analysis. The haematology and blood chemistry analysis were conducted according to the procedure described by Ochei and Kolhatkar (2007).

RESULTS AND DISCUSSION

The results of the chemical composition of SOSM (Table 2) indicated that the raw and processed seed meals have good nutritional properties (20.17 to 23.67% crude protein) and could serve as alternative protein source for albino rats. The diets also indicated adequacy in meeting the nutritional requirements of albino rats in the tropics (Table 3). The crude protein, crude fibre and nitrogen- free extract obtained in this study are close to the values of 23.40, 14.50 and 8.70% reported by Augustine *et al.* (2017a). More nutrient losses were observed in the boiled *Senna obtusifolia* seed meal and this was attributed to the leaching out of these nutrients in boiling water. This observation is in agreement with the report of Nsa *et al*. (2011) and Augustine (2017a) who reported similar findings for boiled castor seeds and *Senna obtusifolia* seeds, respectively. However, an increase in the protein and ash content was observed in the fermented *Senna obtusifolia* seed meal which is in line with the findings of Augustine *et al*. (2018).

The raw *Senna obtusifolia* seeds were observed to contain some anti-nutritional factors (tannins, oxalates, saponins, phytates and total phenols) as presented in Table 4. However, the processing methods were effective in reducing the levels of the anti-nutritional factors. Among the processing methods used, fermentation was observed to be more effective in reducing the levels of the anti-nutritional factors followed by boiling.This finding further concurred with the report of Ikemefuna and Atii (1991) and El-hag *et al.* (2002) who reported that microorganisms in a fermenting medium can breakdown the carbon and nitrogen bond of the anti-nutritional factors and use them for their metabolic activities and in this way denature these metabolites. Towo (2006) also added that rearrangement of the phenolic structure caused by the acidic environment created by the microbes can further denature and deactivate anti-nutritional factors. Augustine *et al.* (2017a) made similar observation for processed *Senna obtusifolia* seeds.

The haematological parameters of the albino rats fed raw or processed SOSM-based-diets are presented in Table 5. The haematological parameters of the albino rats were significantly ($P<0.05$) affected by the dietary treatments. The packed cell volume (PCV), haemoglobin concentration (Hb) and red blood cells (RBC) were significantly ($P<0.05$) depressed in the albino rats fed raw SOSM-based diets. Lower values for these haematological parameters (PCV, Hb, and

Table 1 Ingredient Composition and Calculated Analysis of the Experimented Diets

Ingredient	T1 0%SOSM	T2 20%RSOSM	T3 20%TSOSM	T4 20%BSOSM	T5 20% FSOSM
Maize	49.00	48.00	48.00	48.00	48.00
Maize offal	13.85	7.65	7.65	7.65	7.65
RSBM	17.80	5.00	5.00	5.00	5.00
GNC	16.00	16.00	16.00	16.00	16.00
SOSM	0.00	20.00	20.00	20.00	20.00
Salt	0.30	0.30	0.30	0.30	0.30
Bone meal	2.50	2.50	2.50	2.50	2.50
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.15	0.15	0.15	0.15	0.15
Premix	0.20	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Crude Protein (%)	18.83	18.01	18.11	18.09	18.97
Energy (Kcal/kg)	2911.83	2960.62	2958.23	2948.33	2939.11

Metabolizable energy calculated according to the formula of Pauzenga (1985) expressed as ME = 37x% crude protein + 81x% ether extract + 37.5x% nitrogen-free extract. SOSM = *Senna obtusifolia* seed meal; RSOSM = Raw *Senna obtusifolia* seed meal TSOSM = Toasted *Senna obtusifolia* seed meal; BSOSM, = Boiled *Senna obtusifolia* seed meal and FSOSM = Fermented *Senna obtusifolia* seed meal GNC= groundnut cake, RSBM = Roasted soya bean meal

Table 2 Proximate Composition of Raw or Processed *Senna obtusifolia* Seed Meal

Proximate Components (%)	RSOSM	TSOSM	BSOSM	FSOSM
Dry matter	91.37	90.59	92.01	90.51
Crude protein	22.68	21.97	20.17	23.67
Crude fibre	13.67	11.97	9.67	6.75
Ether Extract (EE)	4.75	3.79	3.21	3.11
Ash	3.67	3.22	2.97	3.32
Nitrogen-free extract (NFE)	39.65	38.85	37.01	37.58
ME (kcal/kg)	2867.36	28002.50	2790.75	2765.31

RSOSM = Raw *Senna obtusifolia* seed meal, BSOSM = Boiled S*enna obtusifolia* seed meal, TSOSM = Toasted S*enna obtusifolia* seed meal and FSOSM = Fermented *Senna obtusifolia* seed meal metabolizable energy calculated according to the formula of Pauzenga (1985), ME = 37 X %CP + 81 X %EE + 35.5 X % NFE. ME= metabolizable energy.

Table 3 Chemical Composition of the Experimental Diets

Proximate components (%)	Level of raw or processed *Senna obtusifolia* seed meal				
	T1 (0%) SOSM	T2 (20%) RSOSM	T3 (20%) TSOSM	T4 (20%) BSOSM	T5 (20%) FSOSM
Dry matter	93.15	92.44	92.45	92.65	91.65
Crude protein	18.01	17.95	18.33	17.57	18.41
Crude fibre	4.25	4.57	4.01	3.98	3.45
Ether extract	7.18	7.01	6.55	6.45	6.40
Ash	6.33	6.50	6.11	6.10	6.60
Nitrogen-free extract	38.45	37.65	37.60	37.52	37.41
Energy (Kcal/kg)	2038.53	2014.74	2019.56	1988.15	2015.63
Tannins	0.003	2.65	1.15	0.65	0.35
Total phenols	0.04	4.35	3.25	3.45	3.05

SOSM = *Senna obtusifolia* seed meal, RSOSM = Raw *Senna obtusifolia* seed meal, BSOSM = Boiled S*enna obtusifolia* seed meal, TSOSM = Toasted S*enna obtusifolia* seed meal, FSOSM = Fermented *Senna obtusifolia* seed meal

Table 4 Levels of Anti-nutritional Factors of Raw or Processed *Senna obtusifolia* Seed Meal

Anti-nutritional factors (%)	RSOSM	TSOSM	BSOSM	FSOSM
Tannins	1.86	0.85	0.43	0.35
Oxalates	0.92	0.63	0.39	0.30
Saponins	1.93	1.01	0.63	0.47
Phytates	3.26	1.15	1.02	0.98
Total phenols	5.25	3.75	4.08	2.25

RSOSM = Raw *Senna obtusifolia* seed meal, BSOSM = Boiled *Senna obtusifolia* seed meal, TSOSM = Toasted *Senna obtusifolia* seed meal and FSOSM = Fermented *Senna obtusifolia* seed meal

Table 5 Haematological Indices of Albino Rats Fed *Senna obtusifolia* Seed meal

	Inclusion levels of *Senna obtusifolia* seed meal					
	T1 0%SOSM	T2 20%RSOSM	T3 20%TSOSM	T4 20%BSOSM	T5 20%FSOSM	SEM
PCV (%)	47.00a	29.67d	31.33c	42.00b	46.67a	1.29
Hb (g/dl)	15.67ab	9.67e	10.55d	12.00c	14.33b	0.58
RBC(x10^6/mm^3	4.13a	2.90c	3.10b	3.53b	3.87b	0.13
WBC (x10^3/mm^3	4.57a	6.77a	5.03b	4.10d	4.60c	0.34

RSOSM = raw *Senna obtusifolia* meal TSOSM = Toasted *Senna obtusifolia* meal; BSOSM = boiled *Senna obtusifolia* meal; FSOSM = fermented *Senna obtusifolia* meal; PCV = packed cell volume; Hb = haemoglobin; RBC = Red blood cells; and WBC = white blood cells.

Table 6 Biochemical Indices of Albino Rats Fed *Senna obtusifolia* Seed Meal Based-Diets

	Inclusion levels of *Senna obtusifolia* seed meal					
	T1 0%SOSM	T2 20%RSOSM	T3 20%TSOSM	T4 20%BSOSM	T5 20%FSOSM	SEM
Total protein (g/dl)	6.54^{a}	3.54^{d}	4.90^{c}	5.33^{b}	5.91^{ab}	1.13
Albumin(g/dl)	3.39	0.91	2.10	2.26	2.41	0.58
Globulin(g/dl)	2.77	2.63	2.80	3.07	3.50	1.47
Creatinine(mmol/L)	65.67bc	106.33a	99.00c	85.00bc	70.00ab	1.63
AST (µ/L)	13.00c	47.33a	36.67b	33.17c	17.67c	0.86
ALT(µ/L)	11.67d	37.33a	36.67b	35.60b	17.67c	3.07
Total bilirubin(mmol/L)	9.67c	16.67a	14.00b	13.00b	10.08c	0.76

RSOSM = raw *Senna obtusifolia* meal TSOSM = Toasted *Senna obtusifolia* meal; BSOSM = Boiled *Senna obtusifolia* meal; FSOSM = Fermented *Senna obtusifolia* meal, AST = Aspartateaminotransferase, ALT = Alanineaminotransferase

RBC) were also observed in the groups of albino rats fed the toasted SOSM-based diets. However, better haematological parameters (PCV, Hb, RBC and WBC) were observed in the albino rats fed 0% SOSM and 20% each of the boiled and fermented SOSM-based diets. White blood cells (WBC) were observed to be significantly high in the group of albino rats fed the raw SOSM-based diets. The depression of the Packed Cell Volume (PCV), haemoglobin (Hb) and Red Blood Cells (RBC) observed in the group of albino rats fed raw SOSM based diets was attributed to the adverse effects of anti-nutritional factors on nutrients required for the production of blood constituents. Nse *et al*. (2014) further reported that nutrients such as protein, vitamins and minerals are required for the production of blood constituents. Therefore, anything that influences nutrient utilization will definitely affect blood constituent The values for PCV, Hb and RBC of the albino rats fed raw SOSM based-diets were observed to be below the normal range of 38.50%, 7.16 x 10^6/ul, 13.60g/dl and 1.98 x 10^3/ul, respectively as reported by Mary and Charlse (2008). This an indication that raw SOSM had negative effects on the blood parameters of albino rats. This finding is in line with the findings of Augustine *et al*. (2017c) who similarly fed albino rats with raw SOSM based-diets and reported depressive effects on their blood constituents. The elevation of the WBC observed in the group of albino rats fed the raw SOSM might be connected to response of the phagocytes to counteract the adverse effects of the anti-nutritional factors as earlier reported by Adedapo *et al.* (2012). The better haematological parameters observed in the group of albino rats fed fermented SOSM-based diets was an indication of the effectiveness of fermentation in detoxifying the anti-nutrients in *Senna obtusifolia* seeds.

The biochemical indices of albino rats fed the experimental diets (Table 6) revealed a significant ($P<0.05$) variation among the treatment means. The albino rats fed the raw and toasted SOSM based-diets indicated lower values for total protein, albumen and globulin. The group of albino rats fed the raw SOSM indicated the least values for total protein, albumen and globulin. The albino rats fed 0% SOSM and 20% each of the boiled and fermented SOSM-based diets on the other hand recorded high values for total protein, albumin and globulin with those fed 0% and 20% fermented SOSM-based diets indicating the highest values. The low values for total protein, albumen and globulin observed in the group of albino rats fed the raw and toasted SOSM-based diets might be attributed to the impact of high concentration of anti-nutritional factors in the raw SOSM and residual anti-nutritional factors in the toasted SOSM. Augustine *et al.* (2017b) also reported similar depressive effect of raw SOSM on the total protein, albumen and globulin of albino rats. This finding corroborates the reports of Soetan and Oyewale (2009) who reported that anti-nutritional factors can inhibit

the activity of digestive enzymes consequently affecting digestion and availability of nutrients.

The urea and creatinine values were observed to be significantly elevated in the group of albino rats fed the raw SOSM-based diets. The values for urea and creatinine of the albino rats fed the raw SOSM-based diets are above the normal ranges of 12.3 to 23.6 mg/dl and 0.2 to 0.5 mg/dl reported by Mary and Charlse (2008), thus indicating adverse effects of the raw SOSM on the kidney of the rats. Lower values for creatinine were recorded in albino rats fed 0 and 20% each of the boiled and fermented SOSM.

The total and conjugated bilirubin levels were significantly ($P<0.05$) higher in albino rats fed the raw and toasted SOSM-based diets. The total and conjugated bilirubin were observed to be lower in albino rats fed 0% and 20% fermented SOSM-based diets compared to other treatment groups. The serum enzymes, alanineaminotransferase (ALT) and aspartateaminotransferase (AST) revealed a similar pattern as that of the total and conjugated bilirubin levels. The ALT and AST were more elevated in the albino rats fed raw SOSM with lower values observed in the albino rats fed 0 and 20% each of the boiled and fermented SOSM-based diets. The values for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of the albino rats fed raw and toasted SOSM-based diets were observed to be above the normal range of 18 to 45, 74 to 143 and 62 to 23U/l, reported by Mary and Charlse (2008). This is an indication that raw and toasted SOSM had some forms of toxicity effects on the liver of the albino rats. The bilirubin levels also indicated similar patterns as that of the serum enzymes. Augustine *et al.* (2017b) made similar observation for albino rats fed raw SOSM-based diets. However, better biochemical values for ALT and AST were observed in albino rats fed the boiled and fermented SOSM-based diets. This implies that boiling and fermentation had reduced the levels of the anti-nutritional factors of SOSM. Generally the biochemical parameters of the albino rats fed the fermented SOSM indicated better values than the other treatment groups.

The biochemical parameters on a general trend indicated evidence of toxicity the group of albino rats fed raw and toasted SOSM. The group of albino rats fed the fermented Senna obtusifolia seed meal based-diet revealed better haematological and biochemical indices. This shows that fermentation was more effective in detoxifying *Senna obtusifolia* seeds than the other processing methods. This concurred with the findings ofAugustine *et al*. (2017a) and Augustine *et al*. (2018).

CONCLUSION

The findings from this study revealed that haematological and biochemical indices were depressed in albino rats fed the raw, toasted and boiled *Senna obtusifolia* seed based-diets with rats fed the raw seed meal base-diet presenting the worst effects. However, better haematological and biochemical values were observed in albino rats fed the processed *Senna obtusifolia* seed meal with those fed the fermented seed meal recording the best values.

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